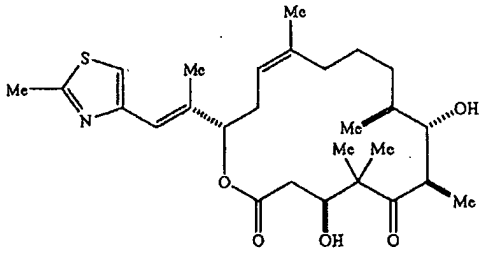
Water and ammonium hydroxide are added and the mixture is stirred for an additional 30 minutes. The reaction mixture is diluted with water and the aqueous phase is extracted with three portions of dichloromethane. The organic phase is then washed with diluted ammonium hydroxide and half-saturated sodium chloride solutions, and evaporated to dryness to afford the crude amino derivative (Derivative 1) functionalized on the thiazole methyl group.

The crude product is purified by column chromatography using silica gel pre-treated with 2.5% methanol-0.2% triethylamine-dichloromethane. The fractions of suitable quality are combined, microfiltered and evaporated to dryness to afford chromatographed Derivative 1. This material is added to ethyl acetate and the resulting suspension is heated at 72–75° C. to obtain a solution. Antisolvent n-heptane is added slowly and the mixture is allowed to cool slowly in the presence of seeds with stirring at 15–25° C. After cooling and holding at ~5° C., the resulting solid is isolated by filtration followed by vacuum drying to afford the purified crystalline amino derivative (Derivative 1) in about 70 M % average yield from Epothilone F.

Example 11

Preparation of Epothilone D (Derivative 3) from Epothilone B



[4S-[4R*,7S*,8R*,9R*,15R(E)]]-4,8-Dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1-oxa-13(Z)-cyclohexadecene-2,6-dione [Epothilone D, Derivative 3].

To anhydrous THF (5 ml) at −78° C. under argon was added WCl_6 (198 mg, 0.5 mmol) followed by nBuLi (0.625 ml of 1.6 M solution in hexanes, 1.0 mmol). The reaction was allowed to warm to room temperature over a 20 minute period. An aliquot (0.50 ml, 0.05 mmol) of the tungsten reagent was removed and added to epothilone B (9.0 mg, 0.018 mmol) under argon and the reaction mixture was stirred for 15 minutes, and then quenched by the addition of saturated $NaHCO_3$ (1 ml). The reaction mixture was extracted with EtOAc (3×1 ml), the combined extracts dried (Na_2SO_4), filtered, and the volatiles were removed under vacuum. The residue was chromatographed with 35% EtOAc/hexanes to give the title compound (7.0 mg, 0.014 mmol). MS m/z 492.3 (M^++H).

What is claimed is:

1. A process for isolation of epothilone B from an epothilone-producing microorganism comprising:

(a) fermenting a strain of epothilone-producing microorganism in the presence of a resin that adsorbs epothilone B by hydrophobic interaction;

(b) collecting the resin in a water-based medium;

(c) extracting the resin with a solvent selected to extract epothilone B and to separate it from the water-based medium; and

(d) crystallizing epothilone B from the extraction phase; wherein said fermentation step further comprises feeding an additive capable of improving the amount of epothilone B produced as compared with the amount of epothilone A produced.

2. The process of claim 1 wherein the crystallized epothilone B from step (d) is substantially pure.

3. The process of claim 1 wherein the resin is extracted with a polar solvent.

4. The process of claim 1 wherein said fermentation step further comprises fermenting said epothilone-producing microorganism in the presence of skim milk, soy flour, yeast extract, maltrin starch, and/or glycerol.

5. The process of claim 1 wherein said fermentation step comprises continuously feeding said additive capable of improving the ratio of epothilone B to epothilone A.

6. The process of claim 1 wherein said additive is a propionic acid salt or ester.

7. The process of claim 6 wherein said additive is sodium propionate, propionic acid methyl ester or propionic acid ethyl ester.

8. The process of claim 1 wherein the crystallization is conducted to reduce the amount of epothilone A to about 55% or less of the amount of epothilone A present after extraction step (c).

9. The process of claim 8 further comprising

(e) at least a second crystallization step effective to reduce the amount of epothilone A to about 55% or less of the amount of epothilone A present after crystallization step (d).

10. The process of claim 1 wherein the epothilone-producing microorganism is *Sorangium cellulosum.*

11. The process of claim 10 wherein said microorganism is *Sorangium cellulosum* strain ATCC No. PTA 3880.

12. The process of claim 10 wherein said microorganism is *Sorangium cellulosum* strain ATCC No. PTA 3881.

13. The process of claim 1 wherein the resin is a styrene/divinylbenzene-based polymer.

14. The process of claim 13 wherein the resin is present in a range of from about 0.2 w/v % to about 5.0 w/v %.

15. The process of claim 1 wherein said step (d) comprises:

(i) adding a second solvent in which epothilone B is either not soluble or sparingly soluble;

(ii) removing at least a portion of the extraction solvent; and

(iii) transitioning the resultant solvent or solvent mixture to a temperature at which epothilone B crystallizes.

16. The process of claim 15 wherein the extraction solvent is ethyl acetate or MTBE, and the second solvent is toluene.

17. The process of claim 1 further comprising:

(f) prior to step (c), washing the resin with aqueous acetonitrile, or aqueous methanol, or an aqueous

medium comprising a detergent and an amine reagent added in base form, the aqueous medium selected to not elute epothilone B.

18. The process of claim 1, wherein step (c) further comprises polish filtering the epothilone B containing solvent.

19. The process of claim 1, wherein epothilone B and epothilone A are produced in an epothilone B/A ratio of at least one.

20. The process of claim 1, wherein epothilone B and epothilone A are produced in an epothilone B/A ratio of at least 1.5.

21. The process of claim 1, wherein epothilone B and epothilone A are produced in an epothilone B/A ratio in the range of 1.5 to 4.0 .

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